

REVIEW

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Mouse models to unravel the role of inhaled pollutants on allergic sensitization and airway inflammation

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Abstract

Air pollutant exposure has been linked to a rise in wheezing illnesses. Clinical data highlight that exposure to mainstream tobacco smoke (MS) and environmental tobacco smoke (ETS) as well as exposure to diesel exhaust particles (DEP) could promote allergic sensitization or aggravate symptoms of asthma, suggesting a role for these inhaled pollutants in the pathogenesis of asthma. Mouse models are a valuable tool to study the potential effects of these pollutants in the pathogenesis of asthma, with the opportunity to investigate their impact during processes leading to sensitization, acute inflammation and chronic disease. Mice allow us to perform mechanistic studies and to evaluate the importance of specific cell types in asthma pathogenesis. In this review, the major clinical effects of tobacco smoke and diesel exhaust exposure regarding to asthma development and progression are described. Clinical data are compared with findings from murine models of asthma and inhalable pollutant exposure. Moreover, the potential mechanisms by which both pollutants could aggravate asthma are discussed.

Introduction

Asthma is a chronic inflammatory disorder of the airways. The clinical hallmark of asthma is bronchial hyperresponsiveness with recurrent episodes of wheezing, breathlessness, chest tightness and cough. These episodes are associated with variable airflow obstruction that is at least partially reversible [1].

Asthma is a considerable public health concern, with an increasing prevalence and an estimate of 300 million asthmatics worldwide. Although the cause of asthma is unknown, there are several risk factors that influence the development of asthma. These can be divided into host factors and environmental risk factors [1]. The allelic distribution of genes pre-disposing to atopy or airway hyperresponsiveness is a typical *host factor* which determines asthma development and phenotype. Typical *environmental factors* are allergens (indoor or outdoor allergens, such as these originating from domestic mites, furred animals, cockroach, fungi, molds, yeasts and

pollen), infections (mainly viruses), occupational sensitizers, tobacco smoke (both active and passive smoking) and indoor or outdoor pollution by gasses and particulate matter (PM) [1,2].

In their efforts to unravel the pathogenesis of asthma, researchers have mainly focussed on the basic immunologic mechanisms resulting in unwanted or exaggerated inflammation. Many uncertainties remain concerning why and how asthma develops during lifetime. The emerging hypothesis is that a failure of endogenous immune regulated tolerance mechanisms might be involved [3]. Alternatively, exposure to a more or less specific cocktail of allergens or pollutants might also lead to the development of an asthmatic phenotype [2]. Regardless of the mechanism, exposure of the airways to foreign agents (allergens or chemical agents) often represents the very first cause for an immune derailment. In later stages, sensitized individuals will be more susceptible to develop airway inflammation and symptoms. These processes can be present for a limited time or become chronic. In that view, the pathogenesis of allergic asthma comprises 3 phases: sensitization, acute inflammation and chronic disease.

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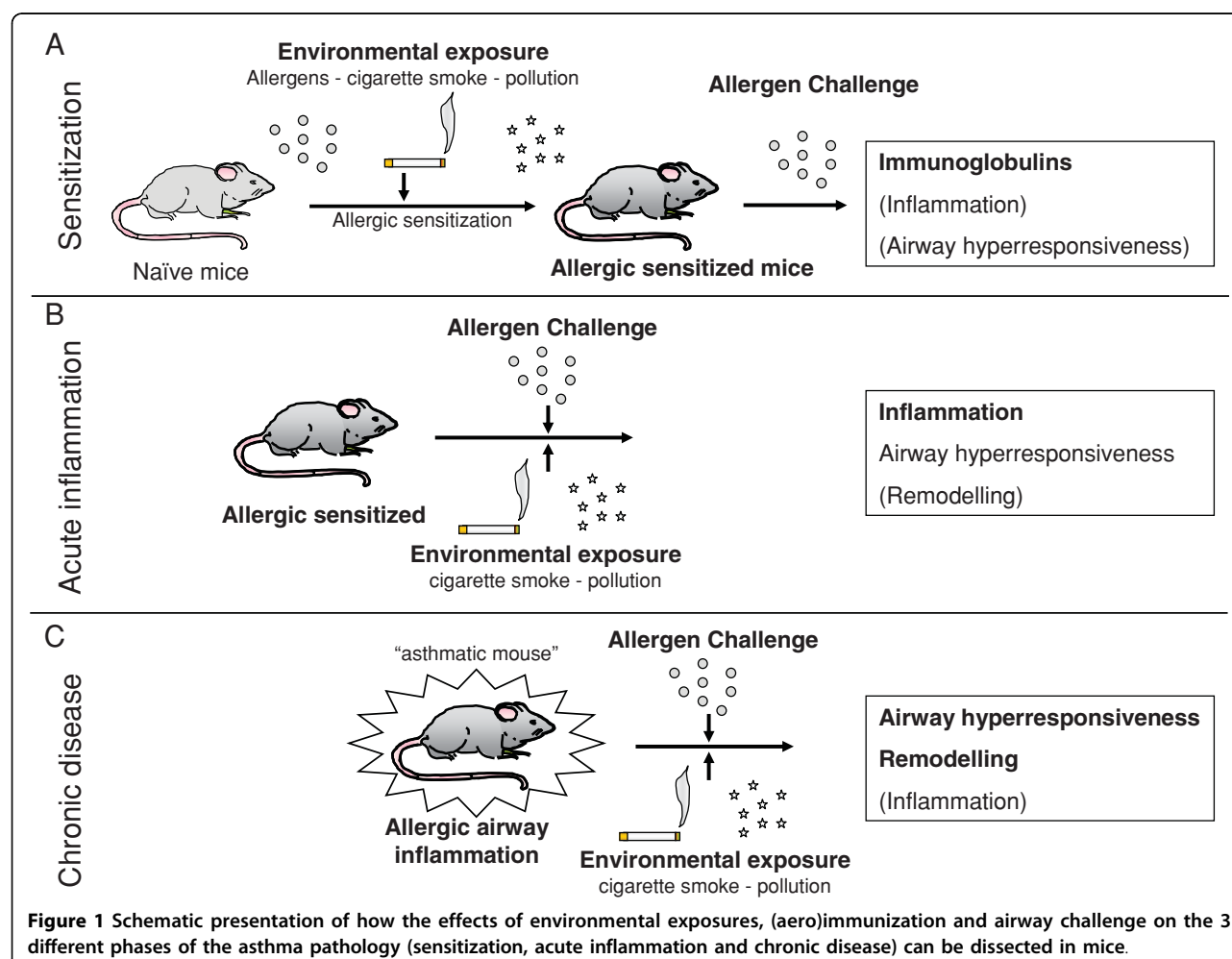
The association between exposure to inhalable pollutants such as cigarette smoke and PM (e.g. diesel exhaust) and respiratory morbidity has been recognized for a long time. The epidemiological association of increased exposure to air pollutants and the rise in frequency of wheezing illnesses led to the assumption that these pollutants are actively involved in the pathogenesis of asthma. While there is no doubt that inhaled pollutants can exacerbate the symptoms of asthma, it is also considerable (though less well established) that they play a role in inducing asthma or at least in driving incipient asthma into clinically obvious manifestations of the disease.

A widely used tool to evaluate the effects of inhaled pollutants on the development and aggravation of asthma consists in epidemiological studies. Controlled exposure studies in humans are informative as well, but are limited by practical and ethical issues. The use of animal models leads to more insights regarding the role of inhalable pollutants during sensitization and inflammation in asthma, with a unique opportunity to unravel

the effects on the different phases of the development of the asthma pathology (Figure 1). The mouse has emerged as the animal of choice for modeling this disease [4]. In this review, we give a summary of the studies investigating the impact of inhaled pollutants on the onset, development or aggravation of asthma. We particularly focussed on tobacco smoke and PM, more specifically diesel exhaust particles (DEP).

Health effects of tobacco smoke and diesel exhaust particles

The World Health Organization (WHO) reports 1.15 billion smokers, of whom 200 million live in Europe [5]. The yearly production of cigarettes still increases in order to meet the people's wishes. Tobacco smoke is a complex mixture of more than 4000 components [6-8]. Researchers distinguish two different emissions from cigarettes. Mainstream smoke (MS) is the smoke actually inhaled by the cigarette smoker (active smoking), whereas side stream smoke is released from the burning end of the cigarette. In many epidemiologic studies, the



term environmental tobacco smoke (ETS) is used, which is a mixture of sidestream smoke and exhaled mainstream smoke in the environment after dilution and aging. This mixed smoke is inhaled during passive smoking (also referred to as "second hand smoke"). Active and passive smoking contribute to the development of various respiratory health problems such as asthma and reduced lung function. In susceptible individuals, active smoking is associated with structural changes in the airways (remodelling, especially of the small airways) and results in destruction of the lung parenchyma (emphysema) [9,10]. Chronic exposure to cigarette smoke induces clinically significant chronic obstructive pulmonary disease (COPD) in 20% of the smokers. Besides COPD, tobacco smoking also causes lung cancer and other adverse health effects [5].

The WHO reports that there is consistent evidence that airborne particulate matter (PM) has a measurable public health impact [11]. The range of health effects is broad, but affects predominantly respiratory and cardiovascular systems. All population is affected, albeit susceptibility to the pollution may vary with age or health status. The risk for various outcomes increases with exposure and adverse effects of PM were demonstrated after both short-term and long-term exposure [11]. Diesel exhaust particles (DEP) are an important component in ambient air pollution and respirable particulate matter. They consist of a carbon core and adsorbed organic substances such as polycyclic aromatic hydrocarbons, and contain small amounts of sulphate, nitrate, metals and other trace elements [12,13]. The majority of diesel exhaust particles are ultrafine particles with a diameter around 0.1 μm , that are highly respirable, reaching the alveoli and the systemic circulation [14]. DEP exposure can induce acute irritation to eyes and throat, light-headedness and nausea, and has been associated with increased respiratory symptoms (cough, phlegm, chronic bronchitis, asthma), increased lung cancer risk and increased risk for total mortality and cardiopulmonary mortality [13-15].

Clinical data on the effect of inhaled pollutants on allergic sensitization and asthma

It is generally accepted that sensitization to allergens is a crucial risk factor for the development of asthma. Studying the potential effects of environmental factors on allergic sensitization is thus relevant. Although the nature of the inhaled particles from cigarette smoke and DEP differs, they induce a similar inflammatory response which is characterized by neutrophils, T-lymphocytes, increased levels of IL-8 and IL-6, along with a decreased phagocytic capacity of alveolar macrophages [12,14,16-19]. One can hypothesize that both cigarette smoke and DEP deploy similar mechanisms, creating an

environment which facilitates allergic sensitization and asthma development.

Epidemiological studies cannot provide the proof that cigarette smoke or DEP exposure are causative factors in the development of asthma. However, associations between the risk of developing asthma and inhalable pollutant exposure provide strong indications that there might be a causal relationship. Many data support the hypothesis that ETS exposure (passive smoking) contributes to the development of both childhood asthma and adult onset asthma [20]. *In utero* exposure to maternal smoking or any smoking at home significantly increases the risk for developing childhood asthma [21,22]. The prevalence of asthma, wheezing, chronic cough and breathlessness in children increases with the number of parents smoking, suggesting a dose-response [23]. ETS exposure during childhood [24] or in adults - mainly occupational exposure - is also associated with the development of adult asthma and other respiratory symptoms [25] in a dose-dependent manner [26]. However, the relationship between ETS exposure and allergic sensitization (as evaluated by serum IgE and skin prick tests) is less evident [27,28]. ETS can promote the induction of Th2 cytokines in nasal fluid of allergic patients, indicative of allergic response exacerbation by ETS in human beings [29]. Passive smoking is indeed dose-dependently related to greater asthma severity, diminished pulmonary function and poorer asthma control in adults [30,31] as well as children [32,33]. The correlation between ETS and asthma prevalence and severity is extensively reviewed in [34].

Contrasting with ETS, the impact of active smoking on the development of asthma is more controversial. Some reports state that active smoking is not a risk factor for adult onset asthma [35], whereas other reports demonstrate the opposite [36,37]. Active smoking during adolescence increases the risk of new onset asthma [38,39]. Asthma prevalence is also higher both in adolescents [40] and among the elderly [41] who smoke. Current wheezing, current asthma and lifetime asthma are all related to active smoking [42]. As for passive smoking, the relationship between active smoking and atopy is again controversial [43,44]. Active smoking is associated with asthma severity, with higher asthma severity scores [35] and less controlled asthma [45]. Smoking asthmatics have a reduced lung function [46], greater decline in FEV1 with age [47] and the lung function is inversely correlated with the amount of cigarettes smoked per day [48]. Active smoking in asthmatics also impairs the therapeutic response to corticosteroids [49].

Many epidemiological data suggest that traffic related air pollution (rather than DEP as such) is a risk factor for wheezing, asthma prevalence and allergic

sensitization (reviewed in [50-52]), however the evidence is not so strong. Nevertheless, several recent birth cohorts demonstrated positive correlations between exposure to traffic pollution and atopic diseases and allergic sensitization in children [53,54]. In contrast to the epidemiological knowledge, experimental approaches have convincingly demonstrated that DEP can facilitate the induction of allergic sensitization. Besides their ability to increase in vivo IgE and cytokine production at the upper respiratory mucosa, DEP can facilitate sensitization to a neoallergen, with the production of allergen-specific IgE and skewing of cytokine production to a T-helper cell 2 pattern [15,55,56]. Ambient air pollution is associated with asthma severity [34,57,58], but reported effects of DEP on aggravation of asthma in controlled exposure studies differ, possibly due to the variety of exposure regimens used in experimental protocols [17,59]. A recent crossover study in London in mild to moderate asthmatics with real life exposure to diesel traffic demonstrated an asymptomatic, though significant reduction in lung function (FEV₁ and FVC), most pronounced in the moderate asthmatics and accompanied by increases in inflammatory markers [60].

Inhaled pollutants and murine allergic sensitization

Mouse models of asthma allow analyses in precisely defined environmental conditions. A commonly used experimental allergen in mouse models is the inert protein ovalbumin (OVA), but also house dust mite, pollen and *Aspergillus* models exist. Sensitization towards OVA, either naturally or upon inhalational exposure, does generally not occur in mice. On the contrary, mice develop inhalational tolerance and become refractory to subsequent immunization attempts by OVA intraperitoneally [61,62]. Some studies intend to break inhalational tolerance by combined exposure regimens in the absence of any intraperitoneal injection, whereas other studies examine the aggravating or modulating effects of inhaled pollutants on the sensitization phase in previously sensitized animals (Figure 1). The impact of tobacco smoke or DEP on allergic sensitization or inflammation in different mouse asthma models will be discussed. In cigarette smoke exposure models, both nose-only and whole body exposures are performed. Side stream smoke is often used as a surrogate for ETS and will be referred to as ETS hereafter. DE(P) models use intranasal or intratracheal DEP-applications or diesel exhaust (DE) inhalation. Additional files 1, 2 and 3 give a detailed overview of methodologies and results from studies with ETS, MS and DEP respectively.

Cigarette smoking and sensitization in mice (Additional files 1 and 2)

Rumold and colleagues proved that **ETS (passive smoking)** can act as an adjuvant for allergic sensitization to OVA [63] (Additional file 1). The co-exposure to ETS and aerosolized OVA induced de novo sensitization, with the development of a memory response [63]. ETS also enhanced allergic sensitization towards intraperitoneal OVA and the effects of ETS were more profound in females, compared to male mice [64,65]. However, in other reports the effects were less clear [66,67]. For example, chronic postnatal exposure to combination of OVA and ETS tended to reduce OVA-specific immunoglobulin production compared to OVA-alone exposure and showed no evident effects on pulmonary inflammation, although airway hyperresponsiveness was increased. Accordingly, ETS-exposure prior to and concomitant with OVA-aerosol exposure could not overcome airway tolerance in three different mouse strains with a different level of susceptibility to airway hyperresponsiveness (A/J, BALB/c and C57BL/6) [67]. Also *in utero* exposure to ETS did not affect antibody production or airway inflammation towards postnatal aerosolized OVA in unsensitized animals, although it did increase airway hyperresponsiveness [68]. ETS exposure prior to, during and after several intranasal sensitizations towards another allergen, *Aspergillus fumigatus* (Af), did not affect IgE-production, but it did increase blood eosinophilia and airway hyperresponsiveness [69]. Thus, despite the absence of IgE markers of sensitization, ETS repeatedly aggravated hyperresponsiveness in different models.

Not all reports are univocal, but some murine models support the hypothesis that ETS can behave as an adjuvant and facilitate allergic sensitization. Although not yet proven, facilitation of allergic sensitization could explain the reported associations between ETS and the increased risk for developing asthma in humans.

In a model mimicking active smoking, in which mice were first exposed for 2-3 months to mainstream cigarette smoke (MS) and subsequently sensitized to OVA or ragweed via the mucosa, smoke exposure increased Th2-cytokine production by splenocytes (suggestive for a heightened allergic sensitization), but attenuated pulmonary inflammation and airway hyperresponsiveness [70] (Additional file 2). In another model without intraperitoneal sensitization, MS could disrupt the normal tolerogenic immune response towards OVA [71]. While OVA aerosol could not induce *per se* any allergic inflammation, simultaneous exposure to OVA and MS induced OVA-specific IgE and IgG₁, pulmonary inflammation and goblet cell hyperplasia [71,72]. In a similar experimental setting, concurrent exposure to MS and OVA induced allergic sensitization with antigen-specific

memory in a GM-CSF dependent fashion [73]. However, prolonged cigarette smoke exposure suppressed eosinophilic inflammation in this model, indicating that cigarette smoke potentially bears both adjuvant and anti-inflammatory properties.

All reports with MS suggest that active smoking can facilitate sensitization in mice, however the data on the subsequent development of allergic inflammation in mice are contradictory. This underscores the need to further elucidate the impact of experimental conditions, which can favour inflammation or, on the contrary, suppress immunity, probably depending on the dose, method and duration of cigarette smoke exposure. Considering that the impact of active smoking on the development of asthma is controversial, these mouse models are very challenging and merit further investigation.

Diesel exhaust particle exposure and sensitization in mice (Additional file 3)

Muranaka and coworkers were the first to show that DEP can increase specific IgE towards OVA or Japanese Cedar Pollen (JCP) after intraperitoneal sensitization [74,75] (Additional file 3). Since then, many authors have described the adjuvant effects of DEP, using different immunization routes. DEP or diesel exhaust can increase OVA-specific IgE, and can increase IL-4 production and cell proliferation in mediastinal and cervical lymph nodes or spleen after intratracheal, intranasal and inhalational sensitization, respectively [76-78]. DEP can thus affect the antigen-specific IgE antibody responses through local and systemic T-cell activation. Similar observations were reported upon sensitization through injection into the footpad [79]. Both the organic matter adsorbed to DEP and the non-extractable carbon core are thought to be responsible for the adjuvant effect [79-81]. Several sensitization models using OVA or house dust mite (Der f) in presence of DEP revealed also increased antigen-specific IgG1 and IgG2 levels, besides increased antigen-specific IgE [81-88]. Moreover, DEP aggravates the observed pulmonary inflammation and goblet cell proliferation in these models.

In line with the experimental data in humans, DE or DEP (self-produced or commercially available reference material) have consistently shown to facilitate allergic sensitization. In contrast to the above mentioned effects of tobacco smoke, biological effects of DEP in mice seem to be less affected by experimental conditions.

Inhaled pollutants and allergen-induced murine asthma models

Different approaches can be used to evaluate the effects of inhaled pollutants on the pathogenesis of allergen-induced airway inflammation.

Firstly, experimental models can evaluate the effect on asthma development. Animals are challenged with allergen in the presence of inhalable pollutants and develop a typical asthmatic phenotype (IgE, pulmonary inflammation, T-cell responses, airway hyperresponsiveness, goblet cell hyperplasia and remodelling) (Figure 1B). The timepoint where the inhalable pollutant is introduced can vary: (1) before the first allergen challenge: assuming that an alteration of the pulmonary environment might induce a higher sensitivity to subsequent allergen challenge; (2) simultaneous exposure: assuming that the presence of the inhalable pollutant and the allergen can affect the pulmonary response to both agents, and in which a possible interaction between both agents can become relevant.

Secondly, models can also evaluate the aggravating effects of inhalable pollutants on mice with previously established allergic airway inflammation, reflecting the human situation of pollutant exposure in existing asthma (Figure 1C).

Additional files 4, 5 and 6 give a detailed overview of methodologies and reported observations in mouse models in which the effects of ETS, MS or DEP exposure on the development or aggravation of asthma were examined.

Cigarette smoking and development or aggravation of asthma in mouse models (Additional files 4 and 5)

Different *in vivo* studies have demonstrated that ETS can aggravate the allergic response in mice which were primed with OVA and had already mounted a Th2 response. Indeed, ETS exposure prior to and during allergen challenge in sensitized mice induces an upregulation of the allergic response, with increased systemic and pulmonary inflammation, which is more pronounced in females compared to males [64,65] (Figure 1B) (Additional file 4). In this experimental setup, the mice, however, also exhibited heightened allergic sensitization (see section on ETS and sensitization), hampering the distinction between effects on sensitization, on asthma development or on both. Enhanced pulmonary inflammation, remodelling and hyperresponsiveness were also observed upon chronic co-exposure to ETS and OVA in "asthmatic mice" (Figure 1C) [89]. *In utero* exposure to ETS has long term effects on the development of allergic inflammation and exacerbates subsequent adult responses to initial allergen exposure [68]. Maternal smoking during pregnancy also induces airway remodelling in mice offspring [90].

In contrast to the reports on ETS, the effects of mainstream cigarette smoke (MS) on the development and exacerbation of allergic inflammation in mice are a matter of debate [91-93] (Additional file 5). Some authors

reported that MS exposure inhibits OVA-induced airway hyperresponsiveness and reduces inflammation in a model of established asthma [70,92]. However, in a BALB/c model examining the development of allergic inflammation, Moerlose et al [93] demonstrated that acute concurrent exposure to allergen (OVA) and MS enhances the allergic pulmonary inflammation, and augments OVA-specific IgE production and airway hyperresponsiveness [93]. These acute effects were confirmed in C57/BL6 mice [94]. Upon prolonged exposures, however, the combination OVA/smoke could delay - though not prevent - the development of tolerance, which is classically observed upon chronic OVA-aerosol exposures [94]. In an "asthmatic mouse", chronic co-exposure to MS and OVA did neither aggravate airway inflammation, OVA-specific IgE production and remodelling, nor accelerate emphysema development [95]. Interestingly, smoke exposure did increase OVA-specific IgE levels in sensitized mice, suggesting that atopic smokers may be at risk for increased allergen-specific IgE, thus increasing their risk for developing asthma [95]. Recently, the importance of the smoke exposure regimens was highlighted, since high dose, but not low dose MS suppressed allergic airway inflammation by inhibiting T-cell function [96].

Complexity of cigarette smoke exposure models

Although it is generally accepted that both active and passive smoking aggravate the severity of asthma in man, murine models suggest the relationship is not that simple. In murine asthma models, there is a discrepancy in the effects of ETS and MS. ETS consistently aggravated all measured outcomes in murine models, similar to observations in humans. MS however, aggravated the development of allergic asthma on the one hand, but it could also suppress established allergic inflammation on the other hand. The origin of the discrepancy between ETS and MS is difficult to define, but can relate to differences in the dose, chemical composition or even particle size of ETS vs MS. Mimicking active smoking in mice is a challenging task and is possibly more subject to variation than ETS exposure. Since mice not "just light a cigarette and smoke", they receive MS by whole body exposure or nose-only exposure. Besides the dose, carbon monoxide levels and stress by the experimental environment (exposure in group vs. individual restrainers) could conceivably impact the immunological response. High doses of cigarette smoke could suppress T-cell or dendritic cell function or induce an increase of blood carboxyhemoglobin levels, which may have immunosuppressive effects on the ensuing allergic inflammation [96,97], whereas low doses of cigarette smoke might promote allergic inflammation. The complexity of effects induced by tobacco smoke exposure is due to its

multipartite nature. Immunosuppressive and anti-inflammatory effects of tobacco smoke are mediated by its oxidants, by carbon monoxide, nicotine and some aromatic compounds that modify transcriptional programmes [98]. Cigarette smoke can moreover chemically modify signalling pathways and extracellular matrix through acetylation, nitrosylation, carbonylation and oxidation which affects cell survival, activation and differentiation [98]. On one hand, smoke exposure can lead to chronic inflammation and damaged respiratory epithelium. On the other hand, tobacco smoking can also acutely suppress epithelial function by increasing permeability and impairing mucociliary clearance. Cigarette smoke can induce infiltration of alveolae by activated macrophages, producing pro-inflammatory mediators, reactive oxygen species and proteolytic enzymes, resulting in inflammation and tissue damage. But, it can also compromise macrophage phagocytic capacity or skew their inflammatory mediator profile [98]. This dual nature of smoke acting on biological processes as both stimulus and suppressor is probably differently reflected in each experimental system, adding to the discrepancies in the reported observations.

In addition, in chronic models with MS or ETS exposure, there is a possibility to obtain phenotypes which overlap with COPD [99-101]. The development of emphysema and airway remodeling for example, which have been reported upon chronic MS exposure [99,100,102] could affect the pulmonary function measurements in an allergic setting. Also lymphoid follicle formation which has been reported in COPD mouse models [103] could contribute to allergic sensitization. This COPD aspect adds to the complexity in interpreting the data, but it can also lead to the development of clinically relevant models of an asthma/COPD overlap syndrome. In any case, further analysis of the animal models and elucidation of the involved mechanisms could provide us with valuable tools to further unravel how tobacco smoke aggravates allergic asthma in humans.

DEP and development or aggravation of asthma in mouse models (Additional file 6)

Besides their effects on allergic sensitization, diesel exhaust particles can enhance the allergen-induced airway inflammation. In most studies, animals are exposed to DEP or diesel exhaust throughout both periods of sensitization and allergen challenge, which renders it difficult to dissect effects on either sensitization or developing airway inflammation. Most reports, however, show that both intratracheal instillation of DEP and inhalation of diesel exhaust increase the allergic response towards OVA or house dust mite in a dose-dependent way with enhanced pulmonary infiltration

and local cytokine production, increased goblet cell hyperplasia, increased airway hyperresponsiveness and, in some strains, increased levels of allergen-specific immunoglobulins [104-112] (Additional file 6).

The aggravating effect of DEP on pre-existent asthma has been examined by exposing previously sensitized and allergen-challenged "asthmatic" mice to DE(P) without further exposure to allergen. In two different models, DE and DEP-exposure clearly increased airway hyperresponsiveness [113,114], but the effects rapidly subsided with continued DE-exposure [113]. The impact on pulmonary inflammation was, however, less pronounced, with no effects of DE(P) on BAL cell numbers and limited effects in the lung.

The above mentioned reports demonstrate that DEP facilitate allergic inflammation and aggravate airway hyperresponsiveness in murine models and correspond with epidemiological data associating particulate air pollution with asthma severity.

Effects of inhaled pollutants in other animal asthma models

In guinea pigs, research focussed mainly on the effects of smoke on airway hyperresponsiveness. Allergen-sensitized animals show an augmented bronchomotor response towards acute MS inhalation compared to non-sensitized animals, which is mediated by endogenous tachykinins [115]. These neuropeptides affect airway smooth muscle tone, vascular permeability, mucus secretion and the release of inflammatory mediators, leading to neurogenic inflammation. Chronic MS exposure significantly increased airway hyperresponsiveness upon allergen challenge in sensitized animals, and upon capsaicin challenge independent of sensitization, indicating that MS can act as an adjuvant for both antigenic and neurogenic airway responsiveness [116].

The effects of DEP on sensitization and the development of allergic airway inflammation were evaluated in Brown Norway rats, using timothy grass pollen, house dust mite or OVA as allergen. As for mice, DEP exposure generally increased the levels of allergen-specific IgE and IgG [117-121]. Also increased eosinophilic airway inflammation [118,119,122] and hyperresponsiveness [118] could be demonstrated, albeit not in all models [117,120,121].

Mechanistic view on the clinical impact of inhaled pollutants on asthma

The proposed mechanisms by which DEP and cigarette smoke favour the allergic sensitization, development and aggravation of asthma have been reviewed previously and are based on reports in mice and man [12,123,124]. Table 1 gives an overview of similarities and differences in mechanistic observations for DEP and cigarette

smoke in both species. The effects of both inhalable pollutants show striking analogies (Figure 2). Inducing damage of the airway epithelium is probably the primary event for both DEP and cigarette smoke. This occurs through direct toxicity or oxidative stress, hereby inducing inflammatory cell recruitment and inflammatory mediator release. Reactive oxygen species (ROS), released directly or indirectly by mononuclear phagocytes, can contribute to airway inflammation through the induction of cytokines, chemokines and adhesion molecules via the NF- κ B pathway and mitogen-activated protein kinase cascades in macrophages and epithelial cells. This inflamed pulmonary environment has the ability to attract dendritic cells and enhance their activation, thereby increasing allergen capture and transport to the lymph nodes [72,125]. Moreover, tobacco smoke and DEP induce epithelial release of the growth factors GM-CSF and thymic stromal lymphopoietin (TSLP), which can stimulate dendritic cell activation [126-129]. Oxidative stress can by itself also affect epithelial cell surface integrity. Co-administration of allergen and inhalable pollutant could thus facilitate penetration of allergen into the epithelial layer, resulting in a more efficient uptake and subsequent antigen presentation by dendritic cells. DEP can adsorb allergens onto their surface and act as carriers to increase allergen deposition into the respiratory tract [130]. This, as well as the decreased phagocytic capacity of alveolar macrophages, could prolong allergen exposure and increase immune reactivity.

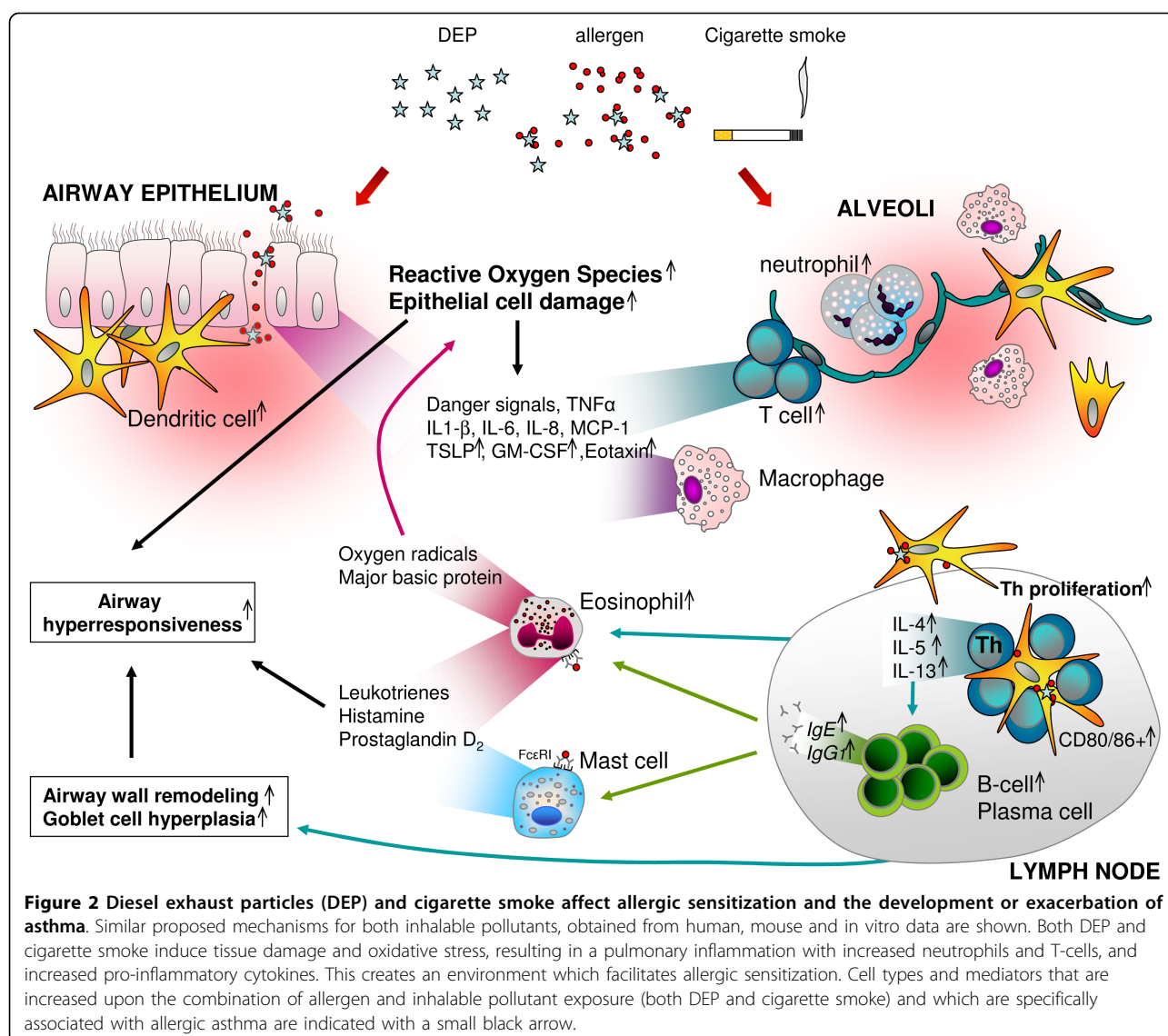
Besides the effects on the innate immune response, DEP or cigarette smoke can affect the adaptive responses towards allergens by enhancing costimulatory molecule expression and T-cell proliferation in the draining lymph nodes [71,72,125,131]. This results in an increased expression of Th2 cytokines, such as IL-4, IL-5 and IL-13. Besides IL-5, also GM-CSF, and eotaxin are increased upon exposure to both inhalable pollutants and allergen. These mediators affect the eosinophil, one of the most prominent cells in the inflammation of allergic asthma, and are responsible for its maturation, survival and attraction to sites of inflammation. IL-4, IL-5 and IL-13 contribute to goblet cell hyperplasia, airway wall remodelling and airway hyperresponsiveness [129]. IgE and IgG production by B-cells is elevated by DEP and cigarette smoke through the action of IL-4. Binding of IgE, crosslinked with allergen, induces eosinophil and mast cell degranulation. The subsequent release of major basic protein and oxygen radicals induces bronchial inflammation, whereas histamine and leukotrienes induce airway hyperresponsiveness, thus further enhancing the effects of both inhaled pollutants on the asthmatic response.

In addition to the above mentioned and established mechanisms, there are newly emerging hypotheses by

Table 1 Mechanistic effects of tobacco smoke and diesel exhaust in human and mouse

TOBACCO SMOKE			DIESEL EXHAUST PARTICLES	
Human	Mouse	BASAL EFFECTS	Human	Mouse
↑[100,144,145]	↑[100,146]	Oxidative Stress/Cell damage	↑* [15,147,148]	↑[15,86,149-151]
↑[18,152]	↑[100,134,146,153,154], f[155]	TNFα	~[156,157]	↑[106,158-161], ↓[107]
↑[18,152]	↑[100,162,163], f [162]	IL-1β	↑* [148], ~[156]	↑[135,150,161], ↓[159]
↑[18,152]	↑[95,163], ~[153]	IL-6	↑[157,164], ~[165]	↑[158,160], f[161]
↑[18,152]	↑[95,100,153,154,163]	IL-8/KC	↑[17,156,166], ↑* [148,167-170], ~[165]	↑[125,135,160]
↑[18,152]	↑[95,100,134,153,154]	MCP-1	↑* [170]	↑[125,150,160], ~[111]
↑, ~[171]	↑ [95], f[171]	GM-CSF	↑* [148,167-169], ~[156,165], f* [128]	↑[172], f[172], ↓[106], ~[104]
~[173]	~[89]	Eotaxin	↑* [174], ~[175]	↑[112], ~[111]
↑[176]	↑[126]	TSLP	↑* [127], f* [127]	▪
↑ [177-179]	↑[134,154,180], ↓[181]	Dendritic cell number	▪	↑[125]
▪	↑[72], ~[181]	DC transport of antigen	▪	↑[125]
↑[177,182]	↑[72,134,180], ↓[181]	Costimulatory molecule expression	↑* [127,128]	↑[84,125]
~or ↑[183,184]	▪	Eosinophil number or degranulation	↑* [185], ~[175,186],	↑[109], ~[106,107,110]
↑[16,18,101,187]	↑[134,153,154,180]	Neutrophil number	↑[15,17,157,175,186,188]	↑[86,104,106,107,110,112,125,158,159]
↑[16,101]	↑[134,153,154,180]	T or B-cell number	↑[15,17,157,175,188], ~[186]	↑[159], ~[104,110]
↑[28,44], ~[25,27]	↑[95], ~[93]	IgE	↑[15,123,189]	~[86,104,106,107,109,110]
↑[190]	~[153]	IgG	~[123,189]	~[86,104,106,107,109,110]
Human	Mouse	EFFECTS IN ALLERGIC DISEASE	Human	Mouse
▪	f [73], ↑[63,73], ~[71]	GM-CSF	↑* [168,169], ~[165]	↑[85], ↓[107]
↑[173]	↑[70,89,93]	Eotaxin	▪	↑[85,88,111,112]
↑[46]	↑[63,72,90], ↓[70,73]	Neutrophil number	↑[166], ~[17]	↑[104-106,108,110,112]
↓[191]	↑[64,72,73,93,94,192], ↓[70,92]	T or B-lymphocyte number	↑[193], ~[17]	↑[84,85,104,105,108-111]
↓[173,194]	↑[63-65,68,69,71-73,89,94], ↓[70,96], ~[92]	Eosinophil number	~[17]	↑[83,85,87,88,104-106,109-111]
▪	↓[96]	T- or B-cell proliferation	↑[193]	↑[76,77,79,84,131]
↓[191]	↑[71-73,93,94], ↓[70]	Dendritic cell number	▪	▪
▪	↑[72]	Costimulatory molecule expression	↑[195]	↑[84,131]
↑[194]	↑[90]	Mast cell number	↑[166]	↑[108]
↑[29]	↑[64,70], ↓[96]	IL-4	↑[55,123,193]	↑[76-78,84,131], ↓ [107]
↑[29], ↓[173]	↑[63,65,68,70,71,73,89], ↓[96]	IL-5	↑[85,123]	↑[85,88,104-107,109-112,131]
↑[29]	↑[65,70,72]	IL-13	↑[123]	↑[112,131]
↑[29,44]	↑[63-65,71,73,93], ~[67-70,90,92,94]	IgE	↑[15,55,56,123]	↑[74-84,86,87,104,105,107,108], ~[106,110]
▪	↑[63-65,71,73], ↓[96]	IgG	↑[56,123], ~[55]	↑[81,82,84-86,88,104,105,107-109,111,112], ~[106,110]
↑[29]	▪	Histamine	↑[15,196]	▪
↑[30,32]	↑[66,68,68,69,89-91,93], ~[67,71], ↓[70,92]	Airway hyperresponsiveness	↑[15,17,59]	↑ [105-108]
↑[194]	↑[89,90], ~[95]	Airway wall remodeling	▪	↑[110]
↑[194]	↑[72,73,89,90], ↓[70]	Goblet cell hyperplasia	▪	↑[83,85,87,106-108,110,111]

▪: not described; ↑: increased; ~: no effect; ↓: decreased; *: in vitro data; f: functional involvement



which inhalable pollutant exposure could affect asthma pathogenesis which merit further investigation [124]. The release of Damage Associated Molecular Patterns (DAMPs) upon inhalable pollutant exposure could play an important role in the asthma pathogenesis. DAMPs are danger signals that can be actively or passively released upon tissue damage or cellular stress. Several of these molecules can stimulate DC maturation and thus act as endogenous adjuvants, such as high mobility group box 1 (HMGB1) protein, heat shock proteins (HSP), adenosine-triphosphate (ATP) and uric acid [124]. The induction of somatic mutations by oxidative DNA damage in lung epithelial barrier cells is also a danger signal that could lead to DC polarization [132]. The activation of the inflammasomes, major intracellular immune response systems that sense danger, is an

interesting pathway for future research [133]. Toll-like receptor signalling, which is activated upon recognition of Pathogen Associated Molecular Patterns (PAMPs) such as LPS, microbial sugars or DNA or RNA is another possible mechanism. For TLR4 it has been demonstrated that it is involved in pulmonary inflammation induced by tobacco smoke and DEP as such [134,135]. In a model of allergic sensitization induced by tobacco smoke exposure, however, both TLR4 and MyD88 appeared not to be required [72]. Recently, a role for TLR9 in an ETS/OVA model was reported [136]. It is of great interest to elucidate the putative pathways further and to determine if and at what level tobacco smoke and DEP show discrepant effects in their ability to induce and aggravate allergic inflammation.

Relevance of data from murine research: pro/con of mouse models

In general, the observed effects of ETS, MS and DEP on the development and aggravation of allergic inflammation in mice correspond well with the observations in asthma patients. The clinical findings for DEP are strongly supported by data obtained in mouse experimental models. In the case of ETS and MS, both clinical findings and mouse data indicate that the relationship between smoke exposure and allergic sensitization and development of clinical asthma is less convincing than for DEP.

Mouse models have several limitations which should be considered when the effects of inhaled pollutant exposure on allergic inflammation are investigated [4,137,138]: i) Mice do not spontaneously develop asthma. ii) The frequently used model-allergen OVA has little biological relevance. However, aggravating effects of pollutant exposure can also be demonstrated in house dust mite models or models without intraperitoneal injections. iii) There are considerable differences in human and mouse immunology [4,137,138]. Species differences in number and size of the alveolar macrophages, for example, can affect the efficiency of alveolar clearance. iiiii) Differences in respiratory physiology and pharmacology can have implications in view of the effects of exposure to inhalational pollutants. Mice are obligate nose breathers, incapable of mouth breathing. The oral breathing in humans bypasses the effective air cleaning capacity of the nose. Mice have lower number of cilia, fewer Clara cells and restriction of submucosal glands to the trachea resulting in a different filtering of inhaled particles compared to man [101]. This can have an impact on the distribution of inhaled particles throughout the respiratory tract [139,140]. Mice furthermore do not have a cough reflex and many mediators such as histamine and tachykinins have different pharmacological effects in humans, which complicates mechanistic analysis of the effects of inhaled pollutants on bronchial hyperresponsiveness [101,138]. iiiiii) Finally, also anatomical and developmental differences can be important. Differences in pulmonary lobulation and bronchial branching (six airway generations in mice versus 23 airway generations in humans), which are already present during the embryonic stage of lung development, can affect particle distribution [139]. In this view, the anatomical location of specific pathological mechanisms induced by particles, such as remodeling, might be different in mice compared to man. In contrast to humans, rodents also do not have respiratory bronchioles. This results in a faster alveolar clearance in rodents, since bronchioles impede rapid clearance of particles from the alveoli.

Species differences may become particularly important in studies on the effects of inhaled pollutant exposure on asthma development early in life. Lung development encompasses different phases of cellular differentiation, branching morphogenesis and overall lung growth, which each can be differently affected depending on the timing of inhaled pollutant exposure [141]. Although the rodent and human respiratory system go across identical phases of development, the timing of each phase is markedly different. In humans, lung growth is essentially complete by the end of adolescence, whereas mouse lungs are more fully developed at birth, implicating that effects of postnatal pollutant exposure in both species cannot be directly compared [142].

However, despite these concerns, rodent models are a valuable tool to test hypotheses which are generated by epidemiological research [4,138] and give more insights in how inhaled pollutant exposure can induce asthma development. i) Mouse models mimic important features of asthma, such as the pulmonary inflammation, remodelling and airway hyperresponsiveness, so the impact of inhaled pollutants on these features can be easily evaluated (Table 1). ii) Important analogies concerning the effects of *in utero* exposures are reported. For example, maternal smoking induces airway remodelling in mice offspring [90], which mimics increased lung remodelling due to *in utero* smoke exposure in children who died from sudden infant death syndrome [143]. Also effects of perinatal ETS exposure on the development of pulmonary function decrements in children can be well modelled in rats [141]. iii) Some important, but more general, advantages of using mouse asthma models are the relatively low cost, the availability of different inbred strains with different immunologic and physiologic properties, the numerous tools for experimental studies, the availability of the complete DNA sequence, and the existence of genetically modified strains [138]. Mice can thus be used for mechanistic studies that are not possible in humans due to ethical reasons. Such mechanistic studies, e.g. using genetically modified mice, are essential for elucidating the contribution of specific mediators or cell types (e.g. dendritic cells).

Mouse models can provide us with a biological basis for the observed associations between air pollution and allergic asthma in humans. They should of course mimic the clinical observations as closely as possible. Distinguishing between sensitization, development and aggravation and carefully selecting the appropriate models to answer specific research questions are therefore essential in studying the impact of inhalable pollutants on the pathogenesis of asthma.

Conclusions

Exposure to inhalable pollutants is an important factor which affects sensitization, development and aggravation of asthma. Although clinical and epidemiological studies provide direct indications about the importance of inhaled pollutants in the pathogenesis of asthma, data from mice hold promise to provide mechanistic clues. We here reviewed the excess of mouse models that are available, focusing on unmet needs that would allow determining the critical mediators involved in the effects of the aforementioned pollutants on the different stages of the disease.

Additional file 1: Table 2: Effects of environmental tobacco smoke (ETS) on murine allergic sensitization. Table 2 provides a detailed overview of methodologies and results from murine models that examine the effects of ETS on allergic sensitization
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Additional file 2: Table 3: Effects of mainstream cigarette smoke (MS) on murine allergic sensitization. Table 3 provides a detailed overview of methodologies and results from murine models that examine the effects of MS on allergic sensitization
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Additional file 3: Table 4: Effects of diesel exhaust particles (DEP) on murine allergic sensitization. Table 4 provides a detailed overview of methodologies and results from murine models that examine the effects of DEP on allergic sensitization
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Additional file 4: Table 5: Effects of environmental tobacco smoke (ETS) on development or aggravation of asthma in murine models. Table 5 provides a detailed overview of methodologies and results from murine models that examine the effects of ETS on development or aggravation of asthma
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Additional file 5: Table 6: Effects of mainstream cigarette smoke (MS) on development or aggravation of asthma in murine models. Table 6 provides a detailed overview of methodologies and results from murine models that examine the effects of MS on development or aggravation of asthma
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Additional file 6: Table 7: Effects of diesel exhaust particles (DEP) on development or aggravation of asthma in murine models. Table 7 provides a detailed overview of methodologies and results from murine models that examine the effects of DEP on development or aggravation of asthma
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Authors' contributions

TM and SP performed the literature search and collected all relevant publications. TM drafted the manuscript. TM and EL designed the figures. GJ, KT and DC assisted with the design of the review. All authors helped to draft the manuscript and critically read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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